Abstracts of Papers

Twenty-eighth Annual Albert L. Tester Memorial Symposium, 16–17 April 2003[1]

The Albert L. Tester Memorial Symposium is held in honor of Professor Albert Tester, who, at the time of his death in 1974, was Senior Professor of Zoology at the University of Hawai'i at Mānoa. The faculty and students of the Department of Zoology proposed an annual symposium of student research papers as a means of honoring, in a continuing and active way, Dr. Tester's lively encouragement of student research in a broad range of fields within marine biology. Papers reporting original research on any aspect of science are solicited from students at the university and these papers are presented at the symposium, which takes place during the spring semester. Income from contributions to the Albert L. Tester Memorial Fund of the University of Hawai'i Foundation is used to provide prizes for the three best papers, judged on quality, originality, and importance of research reported, as well as the quality of the public presentation. Judges include Department of Zoology faculty members and the previous year's student award winners. In addition, a distinguished scholar from another university or research institution is invited to participate in the Symposium as a judge and to present the major Symposium address. In 2003 the distinguished visitor and judge was Dr. William G. Eberhard, Staff Scientist at the Smithsonian Tropical Research Institute and Professor Catedratico at the Universidad de Costa Rica.

Barriers to Gene Flow in the Hawaiian Spinner Dolphin (*Stenella longirostris*)

Kim Andrews[2]

In many cetacean populations, reproductively isolated subgroups exist within populations even when these subgroups live in the same geographic range or are capable of traveling to other subgroups' ranges. The factors that lead to reproductive isolation of these subgroups are often complex and can include such factors as food type and distribution, feeding behavior, social structure, migration patterns, philopatry, and learned behaviors. In the Hawaiian spinner dolphin (*Stenella longirostris*) population, there is variability throughout the Hawaiian Archipelago in geographic distance between suitable habitat, prey distribution, habitat type, availability of habitat, and social structure. To determine whether these factors have led to reproductive isolation, and therefore genetic distinction, between subgroups, I compared genetic structure, movement patterns, and social structure of the Hawaiian spinner dolphin using genetic analyses and available photographic identification data. I collected genetic samples from wild spinner dolphins throughout the Hawaiian Archipelago and sequenced part of the mitochondrial D-loop region to investigate genetic differentiation between dolphins at different islands and between different social groups at islands. Genetic diversity was lower for dolphins at islands with smaller populations and more stable social groups than at islands with larger populations and less stable social groups. In addition, dolphins at the main islands were genetically

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distinct from dolphins at the geographically distant Northwestern Hawaiian Islands. Different social groups inhabiting the same geographic region at Midway Atoll were also genetically distinct. These preliminary results indicate that geographic distance, habitat type, and social structure may be factors that lead to reproductive isolation between subgroups within the Hawaiian spinner dolphin population.

Type Ia Supernovae and the Accelerating Universe[3]

Brian Barris[4]

Five years ago two teams of astronomers revealed evidence indicating that expansion of the universe was accelerating rather than slowing down. The basis for this surprising result was studies of exploding stars known as Type Ia supernovae, which are believed to have a uniform peak brightness (therefore known as "standard candles") that allows their distance to be determined accurately and hence the structure of the universe to be probed. This unexpected discovery has implications for our understanding of the most fundamental physics of the universe and must be subject to extreme scrutiny. In the fall of 2001–2002, a group of astronomers at the University of Hawai'i Institute for Astronomy, along with international collaborators, conducted a new survey for high-redshift Type Ia supernovae using telescopes atop Mauna Kea. Large patches of sky, containing many thousands of galaxies, were observed repeatedly, and after subtracting a template image variable sources were searched for. Sources determined to be good supernovae candidates were subjected to further scrutiny, including spectroscopic follow-up necessary to confirm that they were indeed Type Ia supernovae and thus suitable for cosmological analysis. The survey discovered a total of 14 high-redshift Type Ia supernovae, a substantial addition to existing samples. I have constructed light curves of these objects, determined their distances by light curve analysis, and placed them on a Hubble diagram, which relates distance to redshift. These supernovae agree with previous results, appearing fainter than expected in a universe without acceleration. Though these results may be subject to an as yet unknown systematic effect also affecting prior supernovae studies, they appear to add additional evidence that the universe is indeed undergoing acceleration due to some currently unknown force.

Tracking High-Altitude Birds and Their Diseases on Mauna Kea

Gustav Bodner[5]

Half of Hawai'i's endemic forest birds have gone extinct during the past 150 yr, many from avian malaria and/or avian pox. These diseases are transmitted by mosquitoes that live primarily below 1524 m (5000 ft) in altitude. Despite the importance of elevational movements to the survival of remaining birds, these movements are little known. Using hydrogen isotopes in feathers, we distinguished between 'Amakihi living at 1585 m (5200 ft) and 1890 m (6200 ft) on Mauna Kea. This technique may provide a novel type of population-level information about elevational movements on high oceanic islands.

[3] This project was undertaken with the High-z Supernova Search Team and was partially supported by NASA grant HST GO 09118.09-A.

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Cassiopea ("Upside-down Jellyfish") sp. as an Indicator Species of Water Conditions in Areas on the Windward Side of O'ahu, Hawai'i

Terra Bowen[6]

Cassiopea sp. was first observed in Hawaiian waters around 1941 at Pearl Harbor. Like most invasive species, it is speculated to have been introduced via ballast waters. Since then, *Cassiopea* sp. has been found in "blooms" at a number of other locations around the Islands. The cause of these "blooms" is uncertain; however, much of the literature suggests eutrophication or an increase in nutrient input to the system. Oftentimes, this is due to human interaction in the surrounding environment (e.g., farming, sewage, and residences). This study looked at the populations of *Cassiopea* thriving in areas of anthropogenic changes found on the windward side of O'ahu and their probable link to water eutrophication. Areas with similar properties but without *Cassiopea* sp. populations were also investigated in an effort to determine possible limiting factors for their occurrences. Nutrient content (dissolved and particulate organic matter and inorganic matter), salinity, temperature, turbidity, and oxygen levels were analyzed for each study site. Jellyfish population in these areas was used to determine how effective abundance of *Cassiopea* sp. is as an indicator of water quality.

Size-Frequency Distributions of Coral Populations along West Maui: Rapid Predictors of Reef "Condition"

Eric Brown[7]

Traditional methods to assess and monitor the condition of coral communities are time consuming and costly. Parameters such as coral cover require earlier baseline data for temporal comparisons. A snapshot approach generating colony size-frequency distributions can provide information about the condition or relative trajectory of local coral populations. Over 3000 colonies from 20 species were measured at six sites along the West Maui coastline in 2002. Size-frequency distributions on a normal arithmetic scale demonstrated that reefs were dominated by positively skewed populations of coral species, with most colonies in smaller size classes (< 5000 cm^2). Transforming the distributions using a logarithmic function produced a more normal distribution pattern that was analyzed using statistics such as geometric mean, coefficient of variation (CV), skewness, and mode. Variation in geometric mean colony size was great in some species but not in others, which demonstrated the influence of environmental processes in shaping the size-frequency distribution. Decreasing CV with increasing geometric mean size indicated that long-lived species (i.e., large colonies) had less variation in colony size. This suggests that environmental processes are having a disproportionate impact on the smaller colonies and species. The weak negative relationship between skewness and geometric mean size indicated that smaller colonies were relatively overrepresented in small-sized species and large colonies did not dominate the community distribution at all reefs. Field measurements, data entry, and postprocessing were rapid (~ 3 days) for $n = 500$ colonies per reef in Hawai'i. This method has some limitations, but analyzing size-frequency distributions does appear to be a relatively quick and inexpensive alternative to more conventional techniques for assessing coral populations.

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Reproduction of the Sabellid Polychaete *Sabellastarte spectabilis* in Kāne'ohe Bay, O'ahu, Hawai'i

David R. Bybee[8]

The sabellid polychaete *Sabellastarte spectabilis* occurs in calm, protected waters throughout Hawai'i. It has become one of the most collected marine ornamental species in the state because of its colorful crown and large size. These worms settle among cracks and crevices of delicate reef corals, making collection difficult and potentially damaging to the reef community. Understanding the reproduction and life history of these polychaetes will benefit the marine ornamental trade by facilitating aquaculture of the species and coral reef conservation by decreasing destructive collecting practices. There is little published information on the biology of this species. Experiments to document spawning and larval development were conducted at the Hawai'i Institute of Marine Biology. Four aquariums (20-gallon [75-liter]) were each stocked with 15 worms. Ten worms were randomly selected from two tanks. A portion of the posterior end of each was ablated and they were returned to their respective tanks. Four days after ablation worms in these tanks spawned. No spawning occurred in control tanks. Replication of this experiment 2 months later produced the same results. Eggs are 150–200 μm in diameter, and sperm have a primitive morphology. Cell division in fertilized eggs began approximately 20 min after spawning. Swimming larvae were first seen 7 hr after spawning. Trochophore larvae have a well-developed prototroch and neurotroch. Metatrochophore larvae developed in 4 days with two chaetigers and two types of chaetae. Settlement occurs 7 days after spawning. Larvae were pipetted from the culture system at regular intervals over a period of 10 days and fixed for scanning electron microscopy. Monthly collections sectioned for histological analysis suggest that *Sabellastarte spectabilis* is gonochoristic. This is the first reported induction of spawning and description of larval development from fertilized egg to settlement in this species. It is an important step in the development of technology for artificial propagation and the beginning of establishing knowledge of spawning patterns, fecundity, and larval development in *Sabellastarte spectabilis.*

Habitat Use and Feeding Ecology of the Manta Ray (*Manta birostris*) in Hawai'i

Timothy B. Clark[9]

Manta rays (*Manta birostris*) are large, highly vagile elasmobranchs commonly seen along the Kona coast and have been a major component of the local dive industry for many years. Despite their abundance in Hawai'i, their large size and pelagic nature have made them difficult to study and consequently little is known about their basic ecology. Fisheries for manta rays in Indonesia, Baja California, and the Philippines have increased our need for information on their population structure and habitat use. Preliminary data are presented on the habitat use of manta rays along the Kona coast of the island of Hawai'i. To date, seven manta rays have been tracked using acoustic telemetry. Mantas appear to have a coastal distribution and maintain a home range of between 15 and 30 km over a 3- to 5-day period. Maximum depth recorded was 217 m. Kernal home range analysis suggests that mantas utilize several key areas, and behavioral observations indicate that they may migrate between feeding areas on a daily basis. Mantas usually stay within 1 km of shore during the day; however, at night they mi-

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grate offshore up to 5 km. It is hypothesized that the offshore migration of the mantas is to feed on plankton associated with the deep scattering layer that is migrating upward and inshore during that time.

Socioeconomics of a Hidden Fishery: The Aquarium Fish Industry in West Hawai'i

Jan Dierking[10]

The trade in marine reef fishes for the aquarium hobbyist market is a global $200 million per year industry. The United States is the largest market worldwide. Demand is mostly covered by foreign imports; however, annual collections from reefs on U.S. territory exceed 650,000 fishes. Of these, 70% are caught in Hawai'i. Here, constantly rising catches (to 460,000 fishes in 1997) have caused concern about overfishing and led the State of Hawai'i to close 35.2% of the most important catch area, the Kona coast of the island of Hawai'i, in 2000. Despite the ongoing management effort and high public awareness, reliable industry information is minimal. As part of a study of socioeconomics of reefs in Hawai'i, in 2002 I conducted interviews with half of all active collectors and seven of eight wholesalers in Kona, focusing on industry information (e.g., collection methods and price lists) and collectors' perceptions. Findings include an export ratio for Hawaiian fishes of over 90% compared with official reports of less than 25%, and higher fish prices than previously reported. Results led to a value estimate of the industry of $3.2 million per year, which is four times as much as the most recent official estimate and makes the aquarium fish industry one of the most important fisheries commercially in the state. Notable perceptions of collectors include the belief that their input was not valued in the implementation of protected areas and distrust of the objectivity of state agencies. However, 90% agreed that management is necessary to ensure sustainability. Results indicate that stronger integration of collectors into future resource management efforts may be possible and could benefit conservation of reefs in Hawai'i.

Reconciling Host Association with Phylogeography: Patterns of Genetic Variation in the Tropical Nudibranch Genus *Phestilla*

Anuschka Faucci[11]

Substrate specificity is common among marine invertebrates and often includes chemical settlement cues. Nudibranchs of the genus *Phestilla* occur throughout the tropical Pacific and include seven species, all of which feed and reproduce on specific scleractinian corals. The planktonic larvae of *Phestilla* spp. require a host-specific chemical cue to metamorphose and settle onto their species-specific host coral. *Phestilla sibogae*, *P. lugubris*, *P. minor*, and *Phestilla* sp. 1 occur on species of the genus *Porites*, the dominant reef-building coral in the Hawaiian Islands; *Phestilla* sp. 2 on *Goniopora* spp.; and *P. melanobrachia* on the ahermatypic corals *Tubestrea* spp. A 654 base-pair fragment of the mitochondrial gene *cytochrome c oxidase I* (*COI*) and a 404 base-pair fragment of the ribosomal gene *16S* were sequenced for six species of *Phestilla* from Guam, five species from Palau, and three species from Hawai'i. Maximum-likelihood and maximum-parsimony trees were produced. Most species of *Phestilla* were separa-

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ble based on *COI* and *16S* sequences, and the inferred phylogeographic pattern was complex and highly structured. *Phestilla minor* and *Phestilla* sp. 1 appear to compose a cryptic species complex and cluster according to host corals and geographic locations. Although species of *Phestilla* do not form monophyletic clades according to host corals, there is an apparent relationship between the mtDNA phylogeny and substrate specificity. The inferred molecular phylogenies suggest that host-associated speciation may have played an important role in the evolution of this genus.

Cybersex, the Internet, and Sexuality

Debra L. Golden[12]

Most of the studies currently published on cybersex focus on this new phenomenon as a deviant activity that leads to addiction. To determine attitudes toward cybersex and whether individuals experience any difficulties in using the Internet for recreational and educational sexual activities, an online survey was conducted, with University of Hawai'i undergraduates as participants. Participants were asked to log in to a secure Web site at http://www.goldenservices.net/ to complete a survey entitled Sexual Activities, which consisted of seven basic demographic questions and two attitudinal measures, the Sexual Opinion Survey (SOS) and the Attitudes toward Erotica Questionnaire (ATEQ). The SOS is designed to elicit positive-to-negative effect and reflects the tendency to approach or avoid sexual stimuli. The ATEQ is used to measure attitudes about the positive and harmful effects of erotica, in this case cybersex. The ATEQ also measures whether respondents believe cybersex should be restricted. The amount of time spent online (TSO) and whether participants felt that their TSO interfered with their lives was also measured. Statistical analysis revealed significant (at the 0.05 level) bivariate correlations between scores on the SOS and the ATEQ, demonstrating that general attitudes toward sexual material also seem to apply to cybersex. In addition, results showed that participants were more erotophilic (positive) than erotophobic (negative) in their attitudes toward cybersex, did not consider cybersex particularly harmful or in need of restriction, and reported that TSO was neither excessive nor did it substantially interfere in their lives. Results also showed that participants believed that there could be positive effects of cybersex.

Upwelling of the Equatorial Undercurrent near Three Pacific Equatorial Islands: Implications for Local Ecosystem Processes

Jamison Gove[13]

Oceanographic research was conducted in the Line and Phoenix Islands during the winters of 2000–2002. Temperature plots derived from shallow-water conductivity, temperature, and depth casts (maximum 30 m depth) showed a small pocket of relatively cold water on the west side of three islands, Howland, Baker, and Jarvis Islands. This isolated pocket of water is likely due to the cold, nutrient-rich, eastward-flowing Equatorial Undercurrent impinging on the slopes of these reefs. Comparing hydrographic measurements with biological data, all three islands showed a trend of increased planktivore densities in the upwelled waters. This increase in nutrients stemming from the Equatorial Undercurrent is possibly altering

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the trophic structure of this area, creating unique and isolated biological conditions. El Niño and the Southern Oscillation greatly influence the flow regime of the Equatorial Undercurrent, causing high interannual hydrographic variability. Temperature plots of Baker Island showed a 4 to 5°C difference between La Niña and El Niño events. This variability is likely causing nutrient supply irregularities and has implications for dramatically affecting local ecosystem dynamics.

Pacific Salmon: A Conduit of Marine Nutrients and Organic Matter to Stream Ecosystems

Brittany Graham[14]

Anadromous salmon constitute a biological link between marine, terrestrial, and freshwater environments by delivering vast amounts of marine-derived nutrients annually to freshwater ecosystems. A 2-yr study was conducted in Southeast Alaska to examine the influence of marine-derived nutrients on stream food webs. By utilizing the distinctly elevated ^{13}C and ^{15}N signature of returning salmon, isotopic values of food web components provided a proxy for marine-derived nutrient incorporation. Freshwater fishes, stream invertebrates, and biofilm were collected above and below a salmon migration barrier during pre- and postspawning periods. Invertebrate ^{13}C and ^{15}N values were higher below the barrier after postspawning periods, reflecting marine-derived nutrient incorporation. Prespawning values were also elevated below the barrier relative to above the barrier, demonstrating a legacy effect from previous marine-derived nutrient deposition. The degree of marine-derived nutrient incorporation by stream fishes was a function of age, tissue turnover rate, and migratory behavior. The crucial link between marine-derived nutrient subsidies, via invertebrates, to upper trophic levels appears to be auto- and heterotrophic uptake of mineralized marine-derived nutrients by the biofilm community. Accordingly, grazing invertebrates incorporated marine-derived nutrients rapidly and reflect a longitudinal pattern of nutrient spiraling. This study illustrates that simple food web assumptions cannot be made without examining the intricate pathways of nutrient cycling as revealed by isotope analysis. Furthermore, recent literature has suggested that ^{15}N values be used to estimate salmon escapements levels, but many other factors must be considered before using nitrogen isotopes as a decision-making tool in fisheries management.

Perception of Volcanic Risk in Kona Communities from Mauna Loa and Hualālai Volcanoes, Hawai'i

Chris E. Gregg[15]

Volcanic hazards in Kona (on the western side of the island of Hawai'i) stem primarily from Mauna Loa and Hualālai Volcanoes. Lava flows were emplaced in Kona during 7 of 39 eruptions since 1832, but last impacted Kona in 1950. Hualālai last erupted in ca. 1800. Society's proximity to potential erup-

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tive sources and the potential for fast-moving lava flows, coupled with long time intervals since the last eruptions in Kona, were the underlying stimuli for this study of risk perception. Using questionnaire survey data collected from students and adult residents ($n = 462$), we discuss threat knowledge as an influence on risk perception and perceptions as one driving mechanism for preparedness to protect against volcanic hazards. Knowledge of volcanism on the island is extremely high, but low levels of awareness of both the volcanic threat in Kona and the high velocity with which lava can flow in Kona contribute to low risk perceptions. Respondents exhibited an "unrealistic optimism bias" and inferred that responsibility for community preparedness for future eruptions rests primarily with officials. We infer that these respondents may be less likely to attend to hazard information, react to warnings as directed, and undertake preparedness measures than others who perceive responsibility to lie with themselves. Individual preparedness measures were limited to simple tasks, whereas more involved measures specific to volcanic eruptions were seldom adopted. The findings illustrate the differences in hazard awareness and risk perception between students and adults, between subpopulations representing local areas, and between various ethnicities. Long time intervals since lava flows have occurred in Kona and provision of nonspecific hazard information has contributed to the low levels of preparedness.

Nonrandom Associations of Seabirds with Subsurface Predators in Hawaiian Waters

Aaron Hebshi[16]

Tropical seabirds have long been thought to forage opportunistically on any proper-sized prey within their foraging range. However, feeding specializations could be occurring in ways not previously explored. This study addressed the question of tropical seabird feeding specializations by investigating whether some species in the "tuna-bird" guild in Hawaiian waters prefer to associate with particular species of subsurface predators. Around the main islands, eight species of "tuna birds" are known to feed on prey driven to the surface by tunas, other predatory fishes, and dolphins. If seabirds associate randomly, then for each species the number of individuals observed with a particular subsurface predator would be proportional to the relative abundance of each predator. This study revealed two nonrandom associations: the preference of Wedge-tailed Shearwaters (*Puffinus pacificus*) and Brown Noddies (*Anous stolidus*) to associate more strongly with skipjack tuna (*Katsuwonus pelamis*) than would be expected. A total of 2758 Wedge-tailed Shearwaters was observed with skipjack tuna, significantly greater than the expected 2360 based on a random association. A total of 499 Brown Noddies was observed with skipjack tuna, significantly greater than the expected 435. These feeding specializations may render the populations and ecology of Wedge-tailed Shearwaters and Brown Noddies more sensitive to changes in skipjack tuna abundance than previously believed. Other seabird species observed in association with subsurface predators included White Terns (*Gygis alba*), Sooty Terns (*Sterna fuscata*), Black Noddies (*Anous minutus*), Newell's Shearwaters (*Puffinus newelli*), Red-footed Boobies (*Sula sula*), and Brown Boobies (*Sula leucogaster*); however, insufficient data were collected for these species to test for nonrandom associations.

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The *Fibulin-5* Gene in Cutis Laxa[17]

Qirui Hu[18]

Cutis laxa (CL) is a connective tissue disorder characterized by loose, redundant, and inelastic skin. It was reported that targeted inactivation of the *fibulin-5* gene in mice showed a phenotype similar to that in CL patients. We screened a cohort of 25 unselected CL patients and 2 patients with aortic aneurysms for point mutations in the *fibulin-5* gene (*FBLN5*). By sequencing genomic amplimers from these patients, we found a promoter variant (−152C > T), five intronic variants (IVS1 + 515T > C, IVS1 − 87G > A, IVS1 − 28A > G, IVS2 + 66T > G, IVS10 − 46A > G), two silent alleles (945C > T and 1122C > T), and a nonconservative amino acid substitution (604G > A, G202R). In the group of 184 control individuals, allele frequencies of all the variants were the same as in the CL group, except for alleles IVS1 − 87G > A and G202R, which were not found in normal controls. Southern blot analysis indicated that in most CL patients there were not any chromosome abnormalities affecting *FBLN5*. RNA blot hybridization indicated that there was no significant difference in *FBLN5* mRNA levels between CL and normal cells. However, by immunoblot analysis of extracellular matrix extracts and cell lysates of CL cells we found decreased fibulin-5 levels in cells from patient CL-20, who was heterozygous for variant IVS1 − 87G > A. In the predicted 3D structure of fibulin-5, mutation G202R is located at the hinge between the third and fourth EGF domain. This residue is conserved in fibulin-5 in multiple mammalian species including mouse, rat, and human and therefore it may be important in the folding of fibulin-5 or its interaction with other proteins. Our studies indicate that CL is a genetically heterogeneous disease with a relatively small subset of patients carrying mutations in *FBLN5*.

Importance of Extreme Weather Events to the Breeding Biology of the ʻIʻiwi (*Vestiaria coccinea*)

Wendy A. Kuntz[19]

The ʻIʻiwi (*Vestiaria coccinea*) is an endemic Hawaiian honeycreeper with an extended breeding season (November–June). ʻIʻiwi were once widespread throughout the Hawaiian Islands but are now currently restricted to high-elevation ʻŌhiʻa-Koa rain forest on the major islands. At these high elevations, early breeding pairs are potentially subject to extreme winter weather. Previous researchers have suggested that weather events may contribute to the nest failure rate in several other high-elevation species, but this has not been well documented in a Hawaiian honeycreeper and to date little has been reported about the breeding biology of this species. Here I report on the breeding biology of this species including nest site characteristics and social breeding system. I also report on the impact of storms on both individual and population nest success. The high levels of nest failures following winter storms suggest that extreme weather events may be an important evolutionary factor shaping the breeding system of this species.

[17] Kerstin Wagner, The CL Research Consortium, Hiromi Yanagisawa, and Eric N. Olson are acknowledged.

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Gonad Morphology and Sex Allocation in Sandburrowers (Family Creediidae)

Ross Langston[20]

Sex change has evolved numerous times in marine teleost fishes and is typically accompanied by modifications in gonad morphology during some point in development. These morphologies can be useful in predicting which species change sex as well as pinpointing the origin of sex change within a taxonomic group. In this study I examined patterns of gonad morphology and development in the Creediidae, a family of sandburrowing fishes composed of 16 species allotted in seven genera. My preliminary results from six of these genera indicate that two patterns of gonad development are present within the family: (1) a gonochoristic-type gonad development in which fishes mature as either male or female, have unisexual gonads that are either wholly ovarian or testicular, and do not exhibit evidence of sex change, and (2) a protandric-type gonad development in which juvenile fishes have a bisexual ovotestis, mature initially as males, and change to females with larger size. When transposed onto phylogenetic relationships proposed for the family, these results suggest that the protandric type of gonad development is the derived state for creediids but is secondarily lost in at least two species.

Distinct Roles of Medial Amygdala and Central Amygdala in Unconditioned and Conditioned Fear

Chun-I Li[21]

Preclinical and clinical data suggest that the amygdala plays a role in detection of emotional events and in production of fear responses. The amygdala is composed of distinct nuclei that may serve different functional roles in the modulation of fear. In this study I examined the roles of the medial (MeA) and central (CeA) nucleus of the amygdala in unconditioned and conditioned fear. Following bilateral ibotenic acid lesions of the MeA or CeA, rats were exposed to cat odor, an unconditioned fear stimulus. In comparison with sham-operated controls, rats with MeA lesions exhibited significant deficits in cat odor–induced unconditioned fear as indicated by a significant reduction in the duration of freezing and avoidance and an increase in the frequency of contact with the cat odor stimulus. In contrast, excitotoxic lesions of the CeA had no effects on cat odor–induced unconditioned fear. To examine the role of the MeA and CeA in conditioned fear, rats with similar fiber-sparing lesions of the MeA and CeA were exposed to footshock. Conditioned freezing was measured in the immediate postshock period and a retention test administered after 24 hr. Results indicated that MeA lesions had no effects on contextual fear conditioning as indicated by no significant differences in freezing between lesion and control groups in the immediate postshock period and in the retention test. In contrast, CeA lesions produced significant deficits in freezing occurring in the postshock interval and in the retention test. Together, these results suggest that the MeA, but not the CeA, plays a significant role in the mediation of predator odor–induced unconditioned fear. However, the CeA, but not the MeA, plays an essential role in fear conditioning to a context paired with electric footshock.

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Neural Substrates for Hormone Modulation of Sensory Systems in Reef Fishes

Karen P. Maruska[22]

Neuropeptide hormones released locally in the brain can have profound effects on neural processing and the neural basis of behaviors across taxa. Gonadotropin-releasing hormone (GnRH) is a decapeptide best known for its role in regulation of gonadotropin release from the pituitary. However, extensive GnRH distributions throughout the brain indicate additional functions not directly related to reproductive development. The sensory neuromodulation hypothesis proposes that GnRH released within sensory regions of the brain can influence processing of reproductively relevant sensory information. However, this hypothesis has received little experimental testing in any vertebrate group. Reef fishes are excellent models to examine effects of neuropeptides on sensory-mediated reproduction because they show the greatest diversity of reproductive strategies among vertebrates and use multiple sensory cues to coordinate courtship and spawning. The purpose of this study was to use immunocytochemistry and nerve-labeling techniques to test the primary prediction of the neuromodulation hypothesis that GnRH neurons are located within sensory processing regions of the fish brain. GnRH-immunoreactive cell bodies are found associated with the ganglion of the terminal nerve and the nucleus olfactoretinalis in the forebrain, and in the midbrain tegmentum. Beaded axons from these GnRH cells are located within visual, auditory, and lateral line processing regions of the hindbrain and midbrain in several different coral reef fish species. These data support the first prediction of the neuromodulation hypothesis and identify the neural substrates for peptide modulation of sensory systems. Thus, these results indicate that perception of sensory stimuli can be influenced by neuropeptides, which has important implications for control of reproductive behaviors across all vertebrate taxa.

Evolution of Posterior Hox Genes: Markers for Metazoan Evolution

David Q. Matus[23]

Hox genes are developmental regulatory genes involved in body plan formation and are believed to have diversified before the last common bilaterian ancestor. Most extant organisms possess definitive members from all four primodial bilaterian Hox paralogy classes (anterior, Paralog Group 3, central, and posterior), consistent with an ancestral bilaterian cluster of 8–10 genes. The highly conserved nature of the 60 amino acid homeobox has made Hox genes a useful molecular marker for revealing relationships of several enigmatic phyla, including brachiopods, priapulids, dicyemids, and myxozoans. Orthology assignments of certain Hox genes can be determined by phylogenetic analysis and/or the presence of key diagnostic amino acid motifs. As the number of different taxa surveyed for Hox genes increases, it is apparent that the Hox cluster itself has undergone differing rates of evolution, such that the genes located more 3′ (expressed anteriorally) are more highly conserved, and those expressed more posteriorally are more divergent. For this reason posterior Hox gene evolution has been difficult to reconstruct. Vertebrates possess numerous posterior Hox genes, presumably due to independent duplication events associated with posterior expansion of the Hox cluster. Invertebrates recently have been shown to possess multiple posterior Hox genes, but making one-to-one correspon-

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dences has been difficult. I have isolated, via polymerase chain reaction, posterior Hox genes from representative members of the less-sampled bilaterian groups, including chaetognaths, acoel flatworms, sipunculids, and hemichordates, to provide insight into both the bilaterian ancestral Hox cluster and relationships of enigmatic taxa such as acoels and chaetognaths.

Volcaniclastic Initiation of Karoo Flood Basalt Volcanism[24]

Murray McClintock[25]

Fieldwork in the South Africa and Antarctic sectors of the Karoo Large Igneous Province (LIP) revealed a great diversity of volcanic process operating during the opening stages of flood basalt volcanism beyond simple effusion of lava flows. In many places, the onset of flood volcanism is marked by thick packages of volcaniclastic rocks that are the product of (1) quench fragmentation of lava, (2) injection of fluid basalt into unconsolidated volcaniclastic and country rock, (3) phreatomagmatic explosive eruptions, (4) passive effusion of large volumes of lava, and (5) reworking and mass transport of the products of (1–4). Some of these deposits fill large (25 km^2+) crater complexes cut into preexisting rocks; others blanket prevolcanic topography. Spatial and temporal heterogeneity in availability of external water led to a variety of eruptive styles at different times and depths and at different ephemeral vents within and between these volcanoes, resulting in a complex record of overlapping explosive to effusive volcanism and eolian, fluvial, and volcaniclastic sedimentation. A declining water supply rate and an increasing magma flux as rifting accelerated led to a long-term transition from intense shallow-level intrusion of magma and explosive phreatomagmatic eruptions, the later stages of which are marked by subaerial tuffs and base surge deposits, to voluminous effusion of the Karoo LIP flood basalts. This work also contributes to our growing understanding of how flood basalt volcanism is initiated and evolves with time and how the interplay of sedimentation and volcanism reflects the role of local hydrology and topography in shaping the style of eruption and geometry of resulting deposits.

Diabetes Activates Cell Death Pathway after Ischemic Stroke[26]

Marianna Muranyi[27]

It is well known that hyperglycemia/diabetes aggravates brain damage in experimental and clinical stroke subjects. Hyperglycemia accelerates maturation of neuronal damage, increases infarct volume, and induces postischemic seizures. The mechanism by which diabetes exacerbates ischemic brain damage is still elusive. The objective of this study was to determine whether streptozotocin-induced chronic diabetes activates cell death pathway after cerebral ischemia of 30-min duration. We first determined the pathological outcomes by histopathology and then detected several key components of apoptotic cell death pathway using immunocytochemistry coupled with confocal laser-scanning microscopy and Western blot in hyperglycemic/diabetic and normomoglycemic rats subjected to 30 min cerebral ischemia and followed by 0, 0.5, 3, and 6 hr of reperfusion. The results

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show that cytochrome c was released from the mitochondria to the cytoplasm as early as 30 min after reperfusion and sustained at least 6 hr after reperfusion. Subsequently, caspase-3 was activated and PARP was cleaved. Ligation-mediated polymerase chain reaction on DNA samples detected laddered DNA fragments in hyperglycemic animals. Electron microscopy analysis revealed chromatin condensation, nuclear shrinkage, and marked mitochondrial swelling. It is concluded that activation of apoptotic cell death pathway may contribute to hyperglycemia-exaggerated brain damage and that such aggravated damage has both apoptotic and necrotic components.

Estrogen Differentially Regulates Vitellogenin and Insulinlike Growth Factor-I Production from Primary Hepatocytes in the Tilapia, *Oreochromis mossambicus*

Larry G. Riley[28]

The tilapia, *Oreochromis mossambicus*, exhibits a sexually dimorphic pattern of growth, with males growing faster and larger than females. To investigate the mechanisms regulating the differential growth rates between male and female tilapia, I examined the direct effect of estradiol-17b (E2) and the androgen 5a-dihydrotestosterone (DHT) on the production of insulinlike growth factor-I (IGF-I) and vitellogenin (VTG), precursor to egg yolk protein, using a primary hepatocyte culture. Estradiol significantly stimulated VTG release in a dose-related manner in female hepatocytes, at all doses (0.1–100 mM) tested. In contrast, E2 significantly decreased IGF-I mRNA expression at 10 and 100 mM. When male hepatocytes were used, only the highest dose (100 mM) of E2 elicited a response, increasing VTG and decreasing IGF-I production. In male hepatocytes, DHT significantly increased IGF-I expression in a dose-related manner. It is interesting that in female hepatocytes, DHT at the highest dose (100 mM) stimulated VTG release and inhibited IGF-I expression. This possibly is a result of nonspecific binding of DHT to the estrogen receptor. These data provide strong evidence that estrogens are involved in redirecting metabolic energy from somatic growth (IGF-I production) and toward reproduction (VTG production). Furthermore, the greater growth achieved by males is likely, at least in part, a result of direct androgen stimulation of IGF-I by the liver.

Abiotic versus Biotic Factors Affecting Distribution of Two Coral Species in Ofu Lagoon, American Samoa National Park

Lance Smith[29]

Ofu Lagoon in the National Park of American Samoa is less than 2 m deep, yet over 100 species of stony corals are found in parts of it. Zonation of coral species by abiotic characteristics such as depth, water temperature, substrate, or water motion is not evident, because the distribution of some common coral species across apparently similar habitats is not clearly associated with these parameters. The stony coral species *Pocillopora damicornis* and *Porites cylindrica* are common through parts of the lagoon but absent in others for no readily apparent reason. The objective of this study was to form and test hypotheses regarding the abiotic and biotic factors affecting distribution of these two species in Ofu Lagoon. Thus far, data have been collected at several sites in the lagoon on abiotic characteristics such as water temperature, salinity,

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dissolved oxygen, turbidity, depth, substrate, and water motion, as well as biotic characteristics such as live coral cover, algae cover, and damselfish territory densities. During the next field season, data will be collected for *P. damicornis* and *P. cylindrica* at these sites on coral settlement, coral growth, and predation on corals. Observations thus far suggest that fish predation on corals may be the factor limiting distribution of these two coral species in Ofu Lagoon.

Quantitative Compositional Analysis of Surface Pyroxenes on Mars[30]

Donovan Steutel[31]

Near-infrared spectra of pyroxenes have distinct absorption features at approximately 1 μm and 2 μm, the so-called 1 μm and 2 μm features. The structures of these absorption features—depth, width, and band (wavelength) center—are known to depend on compositions and relative abundances of the minerals clinopyroxene (high-Ca pyroxene) and orthopyroxene (low-Ca pyroxene). I have developed a model to quantify composition of pyroxene-class minerals based on near-infrared spectra. Cations in pyroxenes—calcium, iron, magnesium, and trace elements—affect the band centers of the 1 μm and 2 μm absorption features and do so in different ways in clinopyroxenes and orthopyroxenes. In clinopyroxenes, the band centers are strongly affected by Ca, Fe, and Mg and are at longer wavelengths with increasing Ca and Mg and decreasing Fe. In orthopyroxenes, the band centers depend primarily on Ca and are at longer wavelengths with increasing Ca and Fe and decreasing Mg. I have applied this model to spectra collected by the imaging spectrometer (ISM) instrument that flew aboard the Russian *Phobos* spacecraft in Mars orbit in 1992. My model indicates that the clinopyroxene composition of the surface imaged by ISM is 10–40% Fe and somewhat Ca-rich and the orthopyroxene composition is 0–30% Fe and very Ca-poor. Previous researchers have argued that ISM spectra indicate that the Martian surface resembles that of basaltic shergottites, a class of meteorites known to originate from Mars. However, my results indicate that the orthopyroxenes on the surface of Mars imaged by ISM are too Mg-rich to match a basaltic shergottite composition.

Does Size Matter? Mate Choice in the Monogamous Butterflyfish *Chaetodon multicinctus*

David A. Strang[32]

Sexual selection theory holds that females generally increase their reproductive success by making a larger investment in eggs and care of offspring. Because of this larger investment, they are choosier about their mates than males and are considered to be the limiting sex. Males increase their reproductive success by competing to attract multiple females. In monogamous species, however, the male and female have the same reproductive success, and so both sexes are expected to be choosy. In *Chaetodon multicinctus*, monogamy is enforced by mate guarding, there is no parental care, and there are few opportunities for choice; therefore, we might expect to see a different pattern of mate choice in *C. multicinctus*. I examined mate choice in this species by offering both males and females a choice between two fish of different sizes. A test fish

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was first placed in a long tank and allowed to acclimate. Then two fish of the opposite sex were placed at either end behind a wide mesh barrier. I measured the amount of time the test fish spent at either end of the tank with the stimulus fish. Males spent more time with the larger females, but females spent an equal amount of time with each male. In the wild, males and females mate assortatively. This, however, is not the result of males and females preferring mates that are similar in size. Neither sex showed a preference for the fish that was closer in size. The fact that males are choosier than females suggests two possibilities. One is that both sexes are choosy but that females choose males based on some trait other than size, possibly territory quality. The other is that this is a case of sex role reversal. Males make a larger investment in territorial defense rates and feed less and so might be contributing more to the reproductive success of the pair. This could make them the limiting sex for which females compete.

Bird and Plant Communities in *Acacia koa* reforestation Areas

Laurie Strommer[33]

Forest communities in the Hawaiian Islands have been transformed by human occupation, with declines and extinctions of forest bird populations the result. *Acacia koa*, an endemic codominant tree in wet montane Hawaiian forests, is the current focus of reforestation projects with both ecological restoration and economic goals. I am comparing avian and vegetation communities in two koa reforestation areas with those in nearby native forests with two goals: (1) to determine whether koa reforestation areas grow to resemble native Hawaiian forests in structure, composition, and diversity, and (2) to determine whether and how native forest bird species use the young koa. During the study period I have observed foraging activity in the koa reforestation areas by six native forest bird species, including one endangered species (*Hemignathus munroi*). This compares with eight species that are common in nearby native forest. Preliminary vegetation survey results indicate that, in some cases, koa reforestation, areas develop into forests that resemble native Hawaiian forests in structure and species richness, though not necessarily in species composition and evenness. Although these results are preliminary, they suggest that establishment of *A. koa* plantations may facilitate native ecosystem regeneration on degraded lands though additional efforts may be necessary to ensure development of a diverse understory.

Population Dynamics of Algal Symbionts in the Coral *Montipora capitata*

Junko Toyoshima[34]

The reef-building corals have evolved an elaborated symbiotic relationship with zooxanthellae that plays an important role in supporting productivity and diversity of coral reef ecosystems. In this symbiosis, population density of the symbionts is maintained at an approximately steady level while zooxanthellae cells proliferate and the old cells are constantly replaced by new ones. However, it is unclear how the population is maintained or regulated, although such information may be helpful for understanding coral bleaching (that is, an abnormally low density of zooxanthellae). In plate-shaped colonies of *Montipora capitata* (rice coral), zooxanthellae density is usually different in different locations of a colony. The top layer facing the water column has higher density than the bottom layer facing the substrates. To see

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how the population density is maintained in this coral, the dividing cells were labeled selectively by exposing live colonies to 5-bromo-2′-deoxyuridine (BrdU) for 48 hr. BrdU is an analog of thymidine that is incorporated into nuclear DNA during the usual process of cell division. When visualized with immunohistology, the dividing cells were found in both the top and bottom tissue layers, but in higher proportion in the bottom tissue. Data from long-term tracking of the labeled cells indicate that there is an upward vertical migration of zooxanthellae through the perforate tissue connecting the two layers. This might be the key to protecting the cells from high solar radiation and maintaining the healthy algae population.

Regulation of the N-myc Oncogene in Neuroblastoma Is Dependent on Sp1 and Sp3

Matthew C. Tuthill[35]

Neuroblastoma (nb) is the most common tumor in infants and accounts for 15% of all pediatric deaths from cancer. Amplification and/or overexpression of N-myc correlates with rapid tumor progression and aggressiveness, vascularization, dissemination, advanced stage disease, and poor patient survivorship. Deletion mutagenesis experiments determined that a 26 base-pair retinoic acid response region (RARR) within the proximal N-myc promoter was responsible for mediating the downregulation of N-myc expression upon retinoic acid treatment. Mutation of a CT-box DNA motif contained within the RARR decreased basal transcriptional activity and altered DNA-protein interactions of the promoter, but mutations flanking this motif did not. Both Sp1 and Sp3 transcription factor proteins bound to the wild-type probe as distinct complexes in specifically retarded bands, but neither protein was present on mutated sequences. Lysates from Drosophila S2 cells expressing exogenous Sp1 and Sp3 proteins were able to reproduce the gel shift complexes seen with nb extracts. Transient transfections of S2 cells showed that individually or together, Sp1 and Sp3 were able to trans-activate a N-myc RARR-containing luciferase reporter construct in a dose-dependent manner. Conversely, transfection of the RARR oligonucleotide resulted in a decrease of endogenous N-myc expression in nb cells. In summary, these results suggest that the CT-box element of the N-myc RARR serves a critically functional role and in the basal state allows for N-myc transactivation by Sp1 and Sp3. As a result of this work, we are currently identifying RA-induced modifications in Sp1 and Sp3. In the future, this information has potential utility in the clinical setting to serve as the basis for development of molecular diagnostics in the treatment of nb patients.

Delivery of Foreign DNA into Shrimp Embryos via Electroporation[36]

Nel C. Venzon Jr.[37]

Electroporation has been used successfully for introducing foreign genes in eukaryotic and prokaryotic organisms. This study demonstrates the transient expression of the partial sequence of a *Taura syndrome virus coat protein* (*TSV-CP*) gene in the Pacific white shrimp, *Litopenaeus vannamei*, using optimized electroporation conditions. An expression vector, pBeta-actinP2-TSV-CP-AS, containing the partial sequence of the *TSV-*

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CP gene in anti-sense orientation, has been constructed and introduced into *L. vannamei* embryos (one-cell stage). Optimal electroporation parameters, including voltage, pulse length, and the number of pulses, had been established as 9 volts/cm, 1.20 msec, and 3 pulses, respectively, based on hatching rates. The shrimp embryos were found to be vulnerable to electrical treatment in the first 30 min after spawning. However, we observed some variation in hatching rates among individual shrimp. Transient expression of the *TSV-CP* gene was detected via reverse transcription-polymerase chain reaction (RT-PCR) in *L. vannamei* at the mysis developmental stage. Results of this work show that electroporation is an efficient mode of foreign DNA delivery into embryos of *L. vannamei.*

Utilizing Computer Modeling and Bioinformatics to Develop de novo Proteins as Vaccine Candidates against *Mycobacterium tuberculosis*

Chad B. Walton[38]

Mycobacterium tuberculosis infections account for one-third of the world's total deadly infections, with rates as high as 100 million infected per annum. Tuberculosis (TB) treatment is a complex problem due to a number of factors, including age of initial infection ranging from neonates to elderly, infectious latency that may span 30 or more years, and involvement of both humoral (CD8+) and cell-mediated immunities (CD4+) in the pathogenesis of the disease. To address this complex issue, computer analysis via Hidden Markov Modeling (SAM-T02) and in silico 1D threading (3dPSSMs; protein prediction based on known secondary structure, PSI-Blast, a solvation potential, and an extended structural profile) were coupled to specific bioinformatic prediction to identify native protein motifs that can stimulate each desired immunological effect (via SYFPEITHI, EPITOPE, and Rankpep). The complete *M. tuberculosis* H37Rv genome was scanned and analyzed to identify the candidate regions. Expression of four native proteins (Bfrb, mce1, A85c, and FbpA) containing desired motifs along with two tribrids (Bfrb-A85c-mce1 and Bfrb-A85c-FbpA) is under way for protein purification and subsequent testing as potential vaccine candidates. This work will lead to a structure-function XML (Extensible Markup Language) database that can in turn be utilized for a whole genome analysis based on predicted structure function coupled with in vivo immunological data. The desired net effect is the construction of a computer-based predictive tool for de novo vaccine design.

Group Courtship, Mating Behavior, and Siphon Sac Function in the Whitetip Reef Shark, *Triaenodon obesus*[39]

Nick Whitney[40]

Shark populations are in decline worldwide due to elasmobranchs' vulnerability to fishing pressure. Though an understanding of courtship and mating behavior is crucial to proper management of shark populations, little is known about mating in most species. The goal of this study was to analyze the mating behavior of the whitetip reef shark, *Triaenodon obesus.* We analyzed digital video taken by a collaborator at Cocos Island, Costa Rica, in February 2001. Observations from three separate courtship events include the

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first documentation of group courtship in this species, as well as new evidence regarding the functional mechanics of the siphon sacs. The siphon sacs are paired, ventral, subdermal organs of the male reproductive system that take in seawater before copulation. It has been proposed that these seawater reservoirs serve to flush sperm from previous males out of the female reproductive tract before ejaculation. Our analysis indicates that siphon sacs are used to propel the sperm of the copulating male but not to flush out sperm from previous males. I also discuss the relatively large size and apparent muscular control of the siphon sacs in this species.

Investigation of Sound as a Recruitment Cue in Larval Fishes and *Schindleria* sp.

Amber Whittle[41]

Lack of knowledge concerning the pelagic stage of larval reef fishes is one of the major hurdles being faced in population studies of reef fishes, design of Marine Protected Areas, fisheries management, and other related fields. Experimental data regarding the cues (chemical, visual, auditory) that larval fishes may use to recruit to an area have only been examined in the last 20 yr. I used light and light/sound traps in Kāne'ohe Bay, O'ahu, Hawai'i, to test the usage, by larval fishes, of sound as a recruitment cue. I recorded nocturnal patch reefs sounds and broadcast them using an underwater speaker and an amplifier, a 12V rechargeable battery, and a continuous play tape encased in underwater housing. For both the light and light/sound trap, a collection bucket was located at the bottom of the trap. I set permanent anchor lines over sand bottom and at least 40 m from each other and from surrounding patch reefs. Traps (also included were an empty trap and a purely sound trap, both of which caught nothing), using a randomized square design, were deployed in the spring/summer of 2002, 12 times at dusk and retrieved the following morning. I was surprised to find a significant difference in the catches of nonlarval *Schindleria* sp. in the light and light/sound traps: the purely light traps caught more fishes. I found a substantial difference in the clupeids that I caught. Reef fishes were extremely rare in all my catches. My results indicate that, in Kāne'ohe Bay, nonlarval *Schindleria* sp. and larval clupeids may be deterred from recruiting by nocturnal reef sounds.

Biotic and Abiotic Factors in the Invasion Success of a Caribbean Barnacle in Hawai'i

Chela Zabin[42]

One of the goals of invasion biology is to understand the conditions that make invasions succeed or fail. Although biotic interactions might limit invaders, abiotic processes are also likely to be important. To gain insight into this matter, we studied a number of factors that seemed likely to impact the course of an invasion in Hawai'i. The Atlantic barnacle *Chthamalus proteus* is one of the most abundant recent invaders in the Hawaiian intertidal. Its distribution in Hawai'i is generally limited to protected bays and harbors. Smaller populations are found in areas with moderate wave intensity, where the invader coexists with a number of native and nonnative species. To determine whether biotic interactions might be limiting the barnacle's distribution, laboratory and field experiments were used to examine competition for space with a number of intertidal species. These interactions do not appear strong enough to

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limit the spread of *C. proteus*. Surveys in Curaçao and Panama indicate that *C. proteus* uses similar habitat types in its native range and in Hawai'i. These data suggest that physical factors may limit the spread of the invader and provide some measure of protection for native communities on the open coast. Biotic and abiotic factors may work together to determine the course of an invasion. Outcomes of a competition experiment were different between the first and second years, during which oceanographic conditions changed from El Niño to La Niña. Additional experiments suggest that variation in density of the invader—most likely the result of physical factors—can also change the outcome of competition between *C. proteus* and other barnacles.